

4171-102 CIP

Section I. (Amendments to the Claims)

Please cancel claims 52, 57-61, and 64-65 and amend claims 39, 51, 53-56, 71, 80, and 82, as set out below in the listing of claims 1-83 of the application.

1. (Withdrawn) A method of disinfecting article(s) that are susceptible to contamination by infectious prion protein, the method comprising the steps of:
 - (a) heating said article(s) to a sufficient temperature and for sufficient time to enhance the proteolytic susceptibility of infective prion protein associated with said article(s); and
 - (b) exposing the heated article(s) to a proteolytic enzyme that is effective for at least partial reduction of the infective protein prion associated with said article(s).
2. (Withdrawn) The method of claim 1, wherein said articles comprise surgical instruments.
3. (Withdrawn) The method of claim 2, wherein said surgical instrument(s) are selected from the group consisting of: clamps, forceps, scissors, knives, cables, punches, tweezers, cannulae, calipers, carvers, curettes, scalers, dilators, clip applicators, retractors, contractors, excavators, needle holders, suction tubes, trocars, coagulation electrodes, electroencephalographic depth electrodes, rib and sternum spreaders, bipolar probes, and rib shears.
4. (Withdrawn) The method of claim 1, wherein said article(s) comprise cutleries and kitchen utensils.
5. (Withdrawn) The method of claim 4, wherein said cutleries and kitchen utensils are selected from the group consisting of: knives, forks, scissors, peelers, parers, slicers, spatulas, and cleavers.

4171-102 CIP

6. (Withdrawn) The method of claim 1, wherein said article(s) comprise laboratory apparatus(es).
7. (Withdrawn) The method of claim 6, wherein said laboratory apparatus(es) are selected from the group consisting of: containers, filtration devices, centrifuges, spectrophotometers, and fluorometers.
8. (Withdrawn) The method of claim 1, wherein said article(s) comprise veterinary devices.
9. (Withdrawn) The method of claim 8, wherein said veterinary devices are selected from the group consisting of clamps, forceps, knives, saws, probes, and electronic stun equipment.
10. (Withdrawn) The method of claim 1, wherein the temperature in step (a) comprises a temperature not exceeding about 150°C.
11. (Withdrawn) The method of claim 1, wherein the temperature in step (a) comprises a temperature of at least 35°C.
12. (Withdrawn) The method of claim 1, wherein the temperature in step (a) comprises a temperature below about 150°C.
13. (Withdrawn) The method of claim 1, wherein the temperature in step (a) comprises a temperature in a range of from about 100°C to about 150°C.
14. (Withdrawn) The method of claim 1, wherein the temperature in step (a) comprises a temperature in a range of from about 125°C to about 140°C.
15. (Withdrawn) The method of claim 1, wherein step (b) is conducted at lower temperature than step (a).

4171-102 CIP

16. (Withdrawn) The method of claim 1, wherein step (b) is carried out at temperature above about 40°C.
17. (Withdrawn) The method of claim 1, wherein step (b) is carried out at temperature above about 50°C.
18. (Withdrawn) The method of claim 1, wherein step (b) is carried out at temperature in a range of from about 35°C to about 75°C.
19. (Withdrawn) The method of claim 1, wherein step (b) is carried out at temperature in a range of from about 40°C to about 75°C.
20. (Withdrawn) The method of claim 1, wherein step (b) is carried out at temperature in a range of from about 50°C to about 65°C.
21. (Withdrawn) The method of claim 1, wherein the proteolytic enzyme comprises at least one enzyme selected from the group consisting of keratinase enzymes, proteinase K, trypsins, chymotrypsins, pepsins, chymosins, cathepsins, subtilisins, elastases, collagenases, endopeptidases, peptidases, oligopeptidase, thermolysins, bacillolysin, mycilsins, carboxypeptidases, leucyl aminopeptidases, aminopeptidases, extremothermophilic proteases, carbonyl hydrolase, papain, pancreatin, streptokinase, streptodornase, ficin, carboxypeptidase, chymopapain, and bromelin.
22. (Withdrawn) The method of claim 1, wherein the proteolytic enzyme comprises a keratinase enzyme.
23. (Withdrawn) The method of claim 1, wherein the proteolytic enzyme comprises an active fragment of a keratinase enzyme.

4171-102 CIP

24. (Withdrawn) The method of claim 1, wherein the proteolytic enzyme comprises a *Bacillus licheniformis* PWD-1 enzyme or an active fragment thereof.
25. (Withdrawn) The method of claim 1, wherein the proteolytic enzyme comprises a protease enzyme.
26. (Withdrawn) The method of claim 25, wherein the protease enzyme comprises a carbonyl hydrolase.
27. (Withdrawn) The method of claim 26, wherein the carbonyl hydrolase comprises subtilisin.
28. (Withdrawn) The method of claim 27, wherein the subtilisin comprises a mutant of wild-type *Bacillus amyloliquefaciens* subtilisin, comprising one or more amino acid substitutions, additions, or deletions.
29. (Withdrawn) The method of claim 25, wherein the protease enzyme comprises at least one enzyme selected from the group consisting of: papain, pancreatin, trypsin, chymotrypsin, pepsin, streptokinase, streptodornase, ficin, carboxypeptidase, aminopeptidase, chymopapain, bromelin, and subtilisin.
30. (Withdrawn) A method of removing infective prion protein from a surgical instrument contaminated with same, the method including (a) heating the surgical instrument at a temperature in a range of from about 100°C to about 150°C, followed by (b) exposing the heated surgical instrument to a proteolytic enzyme at a temperature in a range of from about 35°C to about 100°C at which the proteolytic enzyme is thermally stable and proteolytically effective to at least partially destroy the infective prion protein contaminating said surgical instrument.

4171-102 CIP

31. (Withdrawn) The method of claim 30, wherein said heating is conducted for a time of from about 5 minutes to about 5 hours.
32. (Withdrawn) The method of claim 30, wherein the proteolytic enzyme comprises at least one enzyme selected from the group consisting of keratinase enzymes, proteinase K, trypsins, chymotrypsins, pepsins, chymosins, cathepsins, subtilisins, elastases, collagenases, endopeptidases, peptidases, oligopeptidase, thermolysins, bacillolysin, mycilysins, carboxypeptidases, leucyl aminopeptidases, aminopeptidases, extremothermophilic proteases, carbonyl hydrolase, papain, pancreatin, streptokinase, streptodornase, ficin, carboxypeptidase, chymopapain, and bromelin.
33. (Withdrawn) The method of claim 30, wherein the proteolytic enzyme comprises *Bacillus licheniformis* PWD-1 keratinase.
34. (Withdrawn) The method of claim 1, wherein the proteolytic enzyme comprises a protease enzyme.
35. (Withdrawn) The method of claim 34, wherein the protease enzyme comprises a carbonyl hydrolase.
36. (Withdrawn) The method of claim 35, wherein the carbonyl hydrolase comprises subtilisin.
37. (Withdrawn) The method of claim 36, wherein the subtilisin comprises a mutant of wild-type *Bacillus amyloliquefaciens* subtilisin, comprising one or more amino acid substitutions, additions, or deletions.
38. (Withdrawn) The method of claim 34, wherein the protease enzyme comprises at least one enzyme selected from the group consisting of: papain, pancreatin, trypsin,

4171-102 CIP

chymotrypsin, pepsin, streptokinase, streptodornase, ficin, carboxypeptidase, aminopeptidase, chymopapain, bromelin, and subtilisin.

39. (Currently amended) A system for ~~disinfecting articles that are susceptible to contamination by infectious prion protein, said system~~ comprising:

(a) ~~said one or more articles susceptible to contamination by infectious prion protein, characterized by a first temperature of not exceeding about 150°C during a first duration, and a second temperature of at least 40°C during a second, subsequent duration;~~

(b) ~~means for heating said articles during said first duration to said first temperature and for sufficient time to enhance the proteolytic susceptibility of infectious prion protein associated with said articles;~~

(c) a proteolytic enzyme selected from the group consisting of keratinases and subtilisins; and

(d) ~~means for exposing said articles to said proteolytic enzyme at said second temperature during said second, subsequent duration,~~

wherein said one or more articles are characterized by a first elevated temperature of at least 40°C and not more than about 150°C during a first duration, wherein said articles are characterized by a second elevated temperature in a range of from about 50°C to about 65°C and exposure to said proteolytic enzyme during a second, subsequent duration.

40. (Previously presented) The system of claim 39, wherein the proteolytic enzyme comprises keratinase.

4171-102 CIP

41. (Previously presented) The system of claim 40, wherein the keratinase is provided in a solution at a concentration within a range of from about 0.2 g/L to about 1.0 g/L.
42. (Previously presented) The system of claim 41, wherein the solution comprises a solvent selected from the group consisting of distilled water, alcohol, buffer solution, and detergent solution.
43. (Previously presented) The system of claim 42, wherein said solution further comprises one or more chemical additives selected from the group consisting of surfactants, builders, boosters, and fillers.
44. (Previously presented) The system of claim 39, wherein said articles comprise surgical instruments.
45. (Previously presented) The system of claim 44, wherein said surgical instrument(s) are selected from the group consisting of: clamps, forceps, scissors, knives, cables, punches, tweezers, cannulae, calipers, carvers, curettes, scalers, dilators, clip applicators, retractors, contractors, excavators, needle holders, suction tubes, trocars, coagulation electrodes, electroencephalographic depth electrodes, rib and sternum spreaders, bipolar probes, and rib shears.
46. (Previously presented) The system of claim 39, wherein said articles comprise cutleries and kitchen utensils.
47. (Previously presented) The system of claim 46, wherein said cutleries and kitchen utensils are selected from the group consisting of: knives, forks, scissors, peelers, parers, slicers, spatulas, and cleavers.

4171-102 CIP

48. (Previously presented) The system of claim 47, wherein said laboratory apparatuses are selected from the group consisting of: containers, filtration devices, centrifuges, spectrophotometers, and fluorometers.
49. (Previously presented) The system of claim 39, wherein said article(s) comprise veterinary devices.
50. (Previously presented) The system of claim 49, wherein said veterinary devices are selected from the group consisting of clamps, forceps, knives, saws, probes, and electronic stun equipment.
51. (Currently amended) The system of claim 39, wherein said first elevated temperature is higher than said second elevated temperature.
52. (Cancelled).
53. (Currently amended) The system of claim 39, wherein said first elevated temperature is at least about 60°C.
54. (Currently amended) The system of claim 39, wherein said first elevated temperature is in a range of from about 100°C to about 150°C.
55. (Currently amended) The system of claim 39, wherein said first elevated temperature is at least about 75°C.
56. (Currently amended) ~~The system of claim 39, wherein said first temperature is the same as the second temperature~~ A system comprising:
- (a) one or more articles susceptible to contamination by infectious prion protein;
- (b) means for heating said one or more articles;

4171-102 CIP

(c) a proteolytic enzyme selected from the group consisting of keratinases and subtilisins; and

(d) means for exposing said articles to said proteolytic enzyme;

wherein said one or more articles are characterized by an elevated temperature of from about 40°C to about 60°C and exposure to said proteolytic enzyme.

57-61. (Cancelled).

63. (Previously presented) The system of claim 39, wherein the proteolytic enzyme comprises a keratinase enzyme.

64-65. (Cancelled).

66-67. (Cancelled).

68. (Withdrawn) The system of claim 39, wherein the proteolytic enzyme comprises subtilisin.

69. (Withdrawn) The system of claim 68, wherein the subtilisin comprises a mutant of wild-type *Bacillus amyloliquefaciens* subtilisin, comprising one or more amino acid substitutions, additions, or deletions.

70. (Cancelled).

71. (Currently amended) A system ~~for removing infective prion protein from a surgical instrument contaminated with same, the system~~ comprising (a) said a surgical instrument contaminated with infective prior protein, characterized by a first temperature in a range of from about 100°C to about 150°C during a first duration, and a second temperature in a range of from about 35°C to about 150°C during a second, subsequent duration; (b)

4171-102 CIP

means for heating the surgical instrument to ~~said first temperature during said first duration~~; (c) a proteolytic enzyme that is thermally stable at a temperature in a range of from about 35°C to about 100°C and proteolytically effective to at least partially destroy the infective prion protein contaminating said surgical instrument, and (d) means for exposing the surgical instrument to the proteolytic enzyme ~~at said second temperature during the second, subsequent duration, wherein said surgical instrument is characterized by a first elevated temperature in a range of from about 100°C to about 150°C during a first duration, and wherein said surgical instrument is characterized by a second elevated temperature in a range of from about 35°C to about 100°C and exposure to said proteolytic enzyme during a second, subsequent duration.~~

72. (Cancelled).
73. (Previously presented) The system of claim 71, wherein the proteolytic enzyme comprises at least one enzyme selected from the group consisting of keratinase enzymes, proteinase K, trypsins, chymotrypsins, pepsins, chymosins, cathepsins, subtilisins, elastases, collagenases, endopeptidases, peptidases, oligopeptidase, thermolysins, bacillolysin, mycilysins, carboxypeptidases, leucyl aminopeptidases, aminopeptidases, extremothermophilic proteases, carbonyl hydrolase, papain, pancreatin, streptokinase, streptodornase, ficin, carboxypeptidase, chymopapain, and bromelin.
74. (Previously presented) The system of claim 71, wherein the proteolytic enzyme comprises *Bacillus licheniformis* PWD-1 keratinase.
75. (Withdrawn) The system of claim 71, wherein the proteolytic enzyme comprises a protease enzyme.

4171-102 CIP

76. (Withdrawn) The system of claim 75, wherein the protease enzyme comprises a carbonyl hydrolase.
77. (Withdrawn) The system of claim 76, wherein the carbonyl hydrolase comprises subtilisin.
78. (Withdrawn) The system of claim 77, wherein the subtilisin comprises a mutant of wild-type *Bacillus amyloliquefaciens* subtilisin, comprising one or more amino acid substitutions, additions, or deletions.
79. (Withdrawn) The system of claim 75, wherein the protease enzyme comprises at least one enzyme selected from the group consisting of: papain, pancreatin, trypsin, chymotrypsin, pepsin, streptokinase, streptodornase, ficin, carboxypeptidase, aminopeptidase, chymopapain, bromelin, and subtilisin.
80. (Currently amended) A system ~~for disinfecting articles that are susceptible to contamination by infectious prion protein~~, comprising:
- (a) said one or more articles susceptible to contamination by infectious prion protein, ~~characterized by a first temperature in a range of from about 35-150°C during a first duration, and a second temperature in a range of from about 35-100°C during a second, subsequent duration;~~
 - (b) means for heating said articles ~~during said first duration to said first temperature for a sufficient period of time to enhance proteolytic susceptibility of said infective prion protein;~~
 - (c) *Bacillus licheniformis* PWD-1 keratinase; and
 - (d) means for exposing the heated articles to the *Bacillus licheniformis* PWD-1

4171-102 CIP

~~keratinase at said second temperature during said second, subsequent duration,~~

wherein said articles are characterized by a first elevated temperature of at least 40°C and not more than about 150°C during a first duration, and wherein said articles are characterized by a second elevated temperature in a range of from about 50°C to about 65°C and exposure to the *Bacillus licheniformis* PWD-1 keratinase during a second, subsequent duration.

81. (Cancelled).

82. (Currently amended) A system ~~for disinfecting articles that are susceptible to contamination by infectious prion protein~~, comprising:

(a) said one or more articles susceptible to contamination by infectious prion protein; characterized by a temperature in a range of from about 35-100°C;

(b) means for heating said articles;

(c) *Bacillus licheniformis* PWD-1 keratinase; and

~~(b)~~ (d) means for exposing the articles to *Bacillus licheniformis* PWD-1 keratinase,

wherein said articles are characterized by at said an elevated temperature of from about 40°C to about 60°C and exposure to the *Bacillus licheniformis* PWD-1 keratinase for a sufficient period of time to degrade the prion protein.

83. (Cancelled).